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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte NANCY W. Y. HO and ZHENG-DAO CHEN

Appeal 2008-3282
Application 09/180,340
Technology Center 1600

Decided: August 28, 2008

Before LORA M. GREEN, RICHARD M. LEBOVITZ, and FRANCISCO C. PRATS, *Administrative Patent Judges*.

PRATS, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 involving claims to methods and vectors for integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells. The Examiner has rejected the claims as obvious. We have jurisdiction under 35 U.S.C. § 6(b). We affirm-in-part.

STATEMENT OF THE CASE

Claims 14-18, 28-30, and 32-34 are pending and on appeal (App. Br. 2). Claims 14, 29, and 34 are representative of the appealed subject matter and read as follows:

> 14. A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:
>
> (a) transforming the cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, exogenous DNA, and a first selection marker; and
>
> (b) repeatedly replicating the cells from step (a) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, promoting the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA.
>
> 29. A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase flanked on each end by a DNA flanking sequence which is homologous to a reiterated DNA sequence of a target yeast cell, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of the target yeast to form stable integrants which ferment xylose to ethanol.
>
> 34. A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of a target yeast cell, the plasmid further comprising a selection marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating an exogenous DNA sequence into chromosomal DNA of the target yeast cell.

The Examiner applies the following documents in rejecting the claims:

Hallborn[1]	WO 91/15588 A1	Oct. 17, 1991
Ho	WO 95/13362 A1	May 18, 1995

S. Lopes et al., *Factors Affecting the Mitotic Stability of High-copy-number Integration into the Ribosomal DNA of Saccharomyces cerevisiae* YEAST VOL. 12, PP 467-477 (1996).

The following rejections are before us for review:

Claims 14-18, 28-30, and 32-34 stand rejected under 35 U.S.C. § 103(a) as obvious over Ho and Lopes (Ans. 3-7).[2]

Claims 28, 29, and 34 stand rejected under 35 U.S.C. § 103(a) as obvious over Ho and Hallborn (Ans. 7-13).

OBVIOUSNESS -- HO AND LOPES

ISSUE

The Examiner cites Ho as teaching "recombinant yeasts containing genes encoding xylose reductase, xylitol dehydrogenase and xylulokinase, DNA molecules, plasmid vectors and methods useful for producing said yeasts which are capable of fermenting xylose to ethanol and glucose to ethanol" (Ans. 3). The Examiner further cites Ho as teaching "selection markers (pages 15-16) and specific DNA fragments that serve as replicons and selection markers that enable the plasmid to be replicated autonomously in *S. cerevisiae*" (Ans. 4). Regarding the claim limitations requiring the plasmid to be "integrative," the Examiner states:

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[1] The Examiner's Answer mailed September 15, 2005, lists "CA 2090122" as the document number for the Hallborn reference (Ans. 2). However, the document on file having the asserted publication date is WO 91/15588 A1.
[2] Examiner's Answer mailed September 15, 2005.

> In-so-far-as Ho et al. does not explicitly teach the integration at multiple sites, Lopes et al. teach numerous plasmids containing various genes integrated into a RNA gene of S. cerevisiae. Multiple copies of the plasmid were successfully integrated into the genome (over 140 copies), which are stably maintained in non-selective medium for generations over long periods of time (see abstract and pages 467-475). Further, the plasmids contained a Leu2d selection marker and various cloned genes for stability and expression studies.

(Ans. 4.)

Based on these teachings, the Examiner finds that one of ordinary skill in the art would have been prompted to combine the references' teachings "because the method taught by Ho et al. introduces DNA into the same yeast taught by Lopes" (*id.* at 5). Therefore, the Examiner concludes:

> [I]t would have been obvious to one of ordinary skill in the art to arrive at the claimed invention as a whole by combining the teachings of Ho et al. and Lopes et al. because Ho et al. teach the simultaneous fermentation of xylose and glucose into ethanol from the yeast S. cerevisiae, as ethanol is said to be an ideal liquid fuel for automobiles and Lopes et al. teach a method of making transformants stably maintained in non-selective medium for multiple generations over long periods of time.

(*Id.* at 4)

Appellants separately argue three distinct claim groupings. Specifically, Appellants argue that the Examiner erred in finding claims 14-16, 18, 30, 32, and 33 obvious because the Examiner failed to show a teaching or suggestion in the prior art for all of the claimed elements (App. Br. 4-7), and because the Examiner failed to show that one of ordinary skill in the art would have been motivated to practice the claimed invention (*id.* at 7-8). With respect to claims 28 and 34, Appellants argue that the Examiner

failed to show a teaching or suggestion for all of the elements in those claims (*id.* at 8-9). Lastly, with respect to claims 17 and 29, Appellants assert that, given the references' teachings, one of ordinary skill in the art would not have been motivated to practice the invention recited in those claims (*see id.* at 9-11).

We select claims 14, 34, and 29, respectively, as representative of the argued claim groupings. *See* 37 C.F.R. § 41.37(c)(1)(vii). The issue with respect to this rejection, then, is whether the Examiner has established a prima facie case that one of ordinary skill in the art would have considered claims 14, 34, and 29 obvious in view of Ho and Lopes.

PRINCIPLES OF LAW

Recently addressing the question of obviousness, the Supreme Court reaffirmed that under the controlling inquiry, "the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved." *KSR Int' l Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1734 (2007) (quoting *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1, 17-18 (1966)).

> In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art. "[The Examiner] can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references."

In re Fritch, 972 F.2d 1260, 1265 (Fed. Cir. 1992) (citations omitted, bracketed material in original). Thus, as the Supreme Court pointed out in *KSR*, "a patent composed of several elements is not proved obvious merely

by demonstrating that each of its elements was, independently, known in the prior art." *KSR*, 127 S. Ct. at 1741.

While holding that some rationale must be supplied for a conclusion of obviousness, the Court nonetheless rejected a "rigid approach" to the obviousness question, and instead emphasized that "[t]hroughout this Court's engagement with the question of obviousness, our cases have set forth an expansive and flexible approach . . . ." *Id.* at 1739. The Court also rejected the use of "rigid and mandatory formulas" as being "incompatible with our precedents." *Id.* at 1741; *see also* 1742-43 ("Rigid preventative rules that deny factfinders recourse to common sense, however, are neither necessary under our case law nor consistent with it.").

The Court thus reasoned that the analysis under 35 U.S.C. § 103 "need not seek out precise teachings directed to the specific subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ." *Id.* at 1741. The Court further advised that "[a] person of ordinary skill is . . . a person of ordinary creativity, not an automaton." *Id.* at 1742.

Regarding hindsight reasoning, the Court stated that "[a] factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning. Rigid preventative rules that deny factfinders recourse to common sense, however, are neither necessary under our case law nor consistent with it." *Id.* at 1742-1743 (citations omitted).

FINDINGS OF FACT ("FF") PERTINENT TO CLAIMS 14 AND 34

1. Claim 14 recites a method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells. The first step of the

claimed method is transforming the cells with "a replicative and integrative plasmid." The plasmid must have "an autonomous replicating sequence," exogenous DNA, and a first selection marker.

In the second step, the cells from the first step are repeatedly replicated to produce a number of generations of progeny cells while selecting for cells which include the selection marker. Claim 14 provides that the replication step promotes the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produces progeny cells having multiple integrated copies of the exogenous DNA.

2. Claim 34 recites a plasmid vector for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell. The exogenous DNA must be flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of a target yeast cell. The plasmid must also have a selection marker in a position other than between the DNA flanking sequences.

Claim 34 also requires the plasmid to have a "functional yeast autonomous replicating sequence."

3. The Specification does not define "autonomous replicating sequence," or "functional yeast autonomous replicating sequence." However, the Specification states:

> The yeast DNA replication origin, e.g. the ARS [(autonomous replicating sequence)] containing DNA fragment, can be obtained from yeast chromosomal DNA or from chromosomal DNA of other organisms, so long as the DNA fragment can function as an active replication origin to support autonomous replication of plasmid in the host chosen for hcn [(high copy number)] integration. DNA fragments which

> function as ARSs can readily be isolated by incorporating randomly-digested DNA fragments into an E. coli plasmid, followed by transformation of the desired host organism, e.g. a *Saccharomyces yeast*, with the resulting bank of plasmids, as reported in the literature . . . .

(Spec. 27 (citations omitted).)

4. The Specification discloses that when testing candidate sequences for their ability to autonomously replicate in yeast, "[a]ny properly-sized restriction fragments that can make pUCKm6 function effectively as a yeast plasmid must contain an effective 'ARS' and can be used to construct replicative/integrative vectors such as pLNH-ST for high-copy-number integration of exogenous gene(s) into the chromosomes of *S. cerevisiae*" (Spec. 30-31).

5. Ho discloses "genetically engineered yeasts capable of simultaneously fermenting the two major sugar constituents of cellulosic biomass, glucose and xylose, to ethanol" (Ho 1). Ho discloses that such yeasts "can be constructed by cloning a xylose reductase gene, a xylitol dehydrogenase gene, and a xylulokinase gene in yeasts capable of fermenting glucose to ethanol" (*id.*).

6. Ho discloses that the genes encoding the desired xylose-metabolizing enzymes can be delivered to yeast using a plasmid designated as pLSK15 (Ho 15). Ho discloses that:

> pLSK15 . . . is a low copy number plasmid with a copy number of approximately 10 in yeast (S. cerevisiae). It contains the yeast 2μ replicon which enables the plasmid to be replicated autonomously in S. cerevisiae and closely related species. pLSK15 also contains the geneticin (kanamycin) resistance gene (Km^R) and ampicillin resistance gene (Ap^R and also amp^r)

> which serve as selection markers in S. cerevisiae and other yeasts.

(*Id.* at 15-16.)

7. Ho discloses that the genes encoding the desired xylose-metabolizing enzymes can also be delivered to yeast using a plasmid designated as pUCKm10, which "is a high copy-number plasmid (i.e. plasmid with a copy number of about 50 or more) with a copy number close to 100 in S. cerevisiae" (Ho 16). Ho states:

> pUCKm10 is a pUC9 derivative containing the identical 2μ replicon, and the Km^R, and Ap^R genes present in pLSK15. These specific DNA fragments serve as the replicon and selection markers that enable the plasmid to be replicated autonomously in S. cerevisiae (and in related yeasts) and also enable the yeast transformants containing the plasmid to be distinguished from the untransformed host cells.

(*Id.*)

8. Ho does not disclose that its plasmids are "integrative," as required by claim 14. Nor does Ho disclose that its plasmids contain exogenous DNA flanked by sequences homologous to the ribosomal DNA of a target yeast cell.

9. Lopes discloses the development of "a novel type of vector called pMIRY2 (for Multiple Integration in the Ribosomal DNA of Yeast) that integrates into the ribosomal DNA locus of *Saccharomyces cerevisiae* in up to 140 copies which are stably maintained over long periods of growth under non-selective conditions" (Lopes 467).

10. Lopes states that "[i]ntroduction of homologous or heterologous genes by means of [pMIRY2] … led to protein levels similar to those observed using a YEp vector of comparable copy number. Therefore pMIRY2 is a

potentially very useful vector for biotechnological application" (Lopes 467 (citation omitted)).

11. Lopes discloses that pMIRY2 "is basically composed of a 4·5 kb *Bgl*II-B fragment of yeast rDNA (comprising part of the 26S rRNA-gene NTS1, 5S RNA-gene and NTAS2: see Figure 1), a 450 bp chloroplast DNA marker fragment from *Spirodela oligorhiza*, the *LEU*2d gene and pBR322 sequences, still containing the Amp^R-gene" (Lopes 468). When exogenous DNA is put into the plasmid it is flanked by the ribosomal DNA sequences (*see id.* at 469-70).

12. Lopes discloses that "[y]east vectors suitable for high-level expression of heterologous proteins should combine a high copy number with high mitotic stability" (Lopes 467 (abstract)). Lopes discloses, however, that "insertion of a (foreign) gene [into pMIRY2] drastically reduced [the] mitotic stability of the resulting vector in comparison to its parent" (Lopes 467 (abstract)).

13. Lopes investigated the loss of mitotic stability of pMIRY2 by creating a variety of related plasmids, and discovered that

> Plasmid size is an important, but not the only determinant of mitotic stability. Stable maintenance is only observed when the complete plasmid has a size no larger than that of the rDNA unit (9.1 kb). In addition stability depends upon the nature of the rDNA fragment present in the plasmid, required for targeting its integration.

(Lopes 467 (abstract)).

14. With respect to the size requirement of the heterologous DNA within the pMIRY-type plasmids, Lopes specifically discloses:

> Apparently, there is a relatively sharp transition from a mitotically stable to an unstable vector somewhere around a

> size of approximately 9-10 kb, i.e. about the length of an rDNA unit. Even lengthening pMIRY2 by 0.4 kb . . . results in reduction of stability (see above). In conclusion, pMIRY-type vectors are only stably maintained in the rDNA cluster if their size is smaller than or at most equal to the size of the rDNA unit (9.1 kb).

(Lopes 473.)

ANALYSIS – CLAIM 14

Appellants' arguments do not persuade us that the Examiner failed to make a prima facie case that claim 14 would have been obvious to one of ordinary skill in the art in view of Ho and Lopes. We note that Ho does not explicitly disclose the use of an integrative plasmid as recited in claim 14 (FF 8).

However, Lopes discloses that its ribosomal DNA-containing plasmids integrate as many as 140 copies into the yeast rDNA locus, are stably maintained for long periods in yeast (FF 6), and, given the proper configuration (FF 9-11), can result in protein levels that make it useful in biotechnological applications (FF 7). We therefore agree with the Examiner that one of ordinary skill seeking to express the xylose-metabolizing genes taught by Ho would have been prompted to render vectors containing those genes integrative, to achieve the advantages of chromosomally integrating vectors taught by Lopes.

Appellants first argue that the Examiner improperly relied on a document that was not positively recited in the statement of rejection (App. Br. 5). Specifically, Appellants assert that Lopes' statement at page 467, that its pMIRY2 plasmid maintains stability over a long period (FF 6), refers

to a separate document which the Examiner improperly failed to cite (App. Br. 5).

We are not persuaded by this argument. "It is well settled that a prior art reference is relevant for all that it teaches to those of ordinary skill in the art." *In re Fritch*, 972 F.2d 1260, 1264. Appellants point to no evidence of record showing that the statement on page 467 of Lopes is so incorrect or unreliable that one of ordinary skill would ignore it. We therefore do not agree that Lopes' statement should be disregarded.

Appellants argue that the combination of Ho and Lopes fails to teach or suggest all of the limitations of claim 14 because the 2 μm origin of replication of Ho and a yeast autonomous replicating sequence origin "are recognized in the art as different replication origins" (App. Br. 6 (citing Gietz 817)).[3]

We are not persuaded by this argument. It is well settled that during examination, the PTO must interpret terms in a claim using "the broadest reasonable meaning of the words in their ordinary usage as they would be understood by one of ordinary skill in the art, taking into account whatever enlightenment by way of definitions or otherwise that may be afforded by the written description contained in the applicant's specification." *In re Morris*, 127 F.3d 1048, 1054 (Fed. Cir. 1997).

In the instant case, the Specification does not provide a specific definition for "autonomous replicating sequence" as recited in claim 14. However, the Specification does state that "the ARS containing DNA fragment, can be obtained from yeast chromosomal DNA or from

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[3] R. Daniel Gietz et al., *Genetic Transformation of Yeast*, 30 BioTechniques 816-831 (20021

chromosomal DNA of other organisms, *so long as the DNA fragment can function as an active replication origin to support autonomous replication of plasmid in the host*" (Spec. 27 (FF 3) (emphasis added)). Moreover, the Specification discloses that "[a]ny properly-sized restriction fragments that can make pUCKm6 function effectively as a yeast plasmid *must contain an effective 'ARS'* and can be used to construct replicative/integrative vectors" (Spec. 30-31 (FF 4) (emphasis added)).

Given the Specification's use of the term "ARS" as encompassing sequences that allows a vector to function as a yeast plasmid (FF 3, 4), we do not agree with Appellants that the term as used in claim 14 is limited to any particular term of art, nor do we agree that it would be reasonable to exclude the yeast 2 μm origin of replication from the scope of the recitation "autonomous replicating sequence" in claim 14. Thus, Appellants assert that the yeast 2 μm differs from an ARS as the terms are used in the art, but they have not met their burden in clarifying their differences in the context of how "autonomous replicating sequence" is described in the Specification.

Appellants argue that "[t]he plasmids of Ho et al. are able to replicate, but they do not integrate. Thus, they are not replicative and integrative plasmids. Accordingly, Ho et al. do not teach or suggest a method including the use of a replicative and integrative plasmid comprising an autonomous replicating sequence" (App. Br. 6). Appellants similarly argue that, while Lopes' vectors can replicate in E. coli, "the plasmid vectors do not include an autonomous replicating sequence that permits both replication and integration in the same cell. Accordingly, Lopes et al. do not teach or suggest a method including the use of a replicative and integrative plasmid comprising an autonomous replicating sequence" (*id.*). Appellants conclude

that because neither Ho nor Lopes discloses the use of a vector that is both replicative and integrative, "when combined the cited documents do not teach or suggest all the claim limitations of independent claims 14-18 and 30. Thus, the Examiner has failed to present a *prima facie* case that independent claims 14-18 and 30 are obvious in view of the cited art" (App. Br. 7).

We are not persuaded by this argument. It is well settled that "[n]on-obviousness cannot be established by attacking references individually where the rejection is based upon the teachings of a combination of references. . . . [The reference] must be read, not in isolation, but for what it fairly teaches in combination with the prior art as a whole." *In re Merck & Co., Inc.*, 800 F.2d 1091, 1097, 231 USPQ 375, 380 (Fed. Cir. 1986).

Therefore, the teachings of the individual references may not be viewed alone, but instead must be viewed alongside the teachings of the other reference. In the instant case, in the context of delivering exogenous DNA to yeast, Ho teaches the desirability of using plasmids containing a yeast autonomous replication sequence in order to maintain an adequate intracellular copy number (*see* FF 6, 7), and Lopes teach that using ribosomal DNA integrating sequences allows for mitotically stable integration of exogenous DNA into the yeast chromosome (FF 9, 10, 13). Given Lopes' disclosure of the desirability of both high copy number and mitotic stability (FF 12), we agree with the Examiner that one of ordinary skill in the art would have been prompted to combine the two approaches so as to meet those objectives when delivering the xylose-metabolizing genes. We are therefore not persuaded by Appellants that the cited combination of references fails to suggest all of the limitations in claim 14.

Appellants argue that the cited prior art fails to provide motivation for practicing the process of claim 14 (App. Br. 7-8). Specifically, Appellants assert that "[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination" (*id.* at 8 (quoting M.P.E.P. § 2143.01)). Moreover, Appellants urge, "the use of the same yeast in each cited document does not provide any suggestion as to the desirability of making the combination." (*id.*) Thus, Appellants conclude, "the Examiner has not provided any motivation to combine the cited documents, and therefore the Examiner has failed to present a *prima facie* case that claims 14-16, 18, 28-30, and 34 are obvious in view of the cited art" (*id.*).

We are not persuaded by this argument. The obviousness analysis is not limited to the explicit statements made in the prior art, but must take into account "the inferences and creative steps that a person of ordinary skill in the art would employ" based on the references' teachings. *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. at 1741.

In the instant case, for the reasons discussed above, we agree with the Examiner that, being a person of ordinary creativity and common sense, *KSR*, 127 S. Ct. at 1742-43, one of ordinary skill in the art seeking to express the xylose-metabolizing genes taught by Ho would have been prompted to render vectors containing those genes integrative, to achieve the advantages of chromosomally integrating vectors taught by Lopes. We therefore do not agree with Appellants that the Examiner failed to make a prima facie case with respect to claim 14.

Thus, we affirm the Examiner's obviousness rejection of claim 14 over Ho and Lopes. Because claims 15, 16, 18, 30, 32, and 33 were not

argued separately from claim 14, they fall with that claim. 37 C.F.R. § 41.37(c)(1)(vii).

ANALYSIS – CLAIM 34

Appellants present separate argument with respect to claims 28 and 34. As noted above, we select claim 34 as representative of the argued claim grouping. 37 C.F.R. § 41.37(c)(1)(vii).

Appellants' arguments do not persuade us that the Examiner failed to make a prima facie case that one of ordinary skill in the art would have considered claim 34 obvious in view of Ho and Lopes. As discussed above, we agree with the Examiner that one of ordinary skill would have considered it obvious to combine the approaches of Lopes and Ho to prepare a plasmid having a yeast autonomous replicating sequence as taught by Ho (FF 6, 7), and rDNA sequences flanking as taught by Lopes (FF 9-11) to deliver Ho's xylose-metabolizing genes to yeast.

Moreover, given the Specification's apparently more general use of the term "ARS" as encompassing sequences that allow a vector to function as a yeast plasmid (FF 3, 4), we do not agree with Appellants that the term "yeast autonomously replicating sequence" in claim 34 is limited to any particular term of art, nor do we agree that it would be reasonable to interpret claim 34 as excluding the yeast 2 μm origin of replication disclosed by Ho.

Thus, Appellants' arguments do not persuade us that the cited references fail to teach or suggest all of the claimed limitations. We therefore affirm the Examiner's rejection of claim 34. Because claim 28 was argued in the same group as claim 34, it falls with claim 34. *See* 37 C.F.R. § 41.37(c)(1)(vii).

FINDINGS OF FACT PERTINENT TO CLAIM 29

15. Claim 29 recites a plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of the target yeast to form stable integrants which ferment xylose to ethanol. The plasmid must have a functional yeast autonomous replicating sequence and exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated DNA sequence of a target yeast cell.

The exogenous DNA in the plasmid must include genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase.

16. To prepare plasmids capable of conferring xylose-metabolizing ability to yeasts, Ho discloses that the "XR [(xylose reductase)], XD [(xylose dehydrogenase)], and XK [(xylulokinase)] genes fused to the proper promoters were . . . cloned on pLSK15 . . . or pUCKm10" (Ho 15).

16. Example 2 of Ho discloses the fusion of the pyruvate kinase promoter to the xylitol dehydrogenase (XD) gene (Ho 21-22). Ho discloses that the construct "contains -910 to -1 promoter fragments from the pyruvate kinase gene and +1 to +1963 nucleotides from the Pichia XD gene" (*id.* at 22).

17. Example 3 of Ho discloses that the xylulokinase gene used by Ho to transform yeast is about 2.4 kb, with the translated region containing 2118 nucleotides (Ho 22).

18. The combined length of the translated regions of Ho's xylulokinase and xylitol dehydrogenase genes is therefore 1.9 kb (XD) plus 2.1 kb (XK), or about 4.0 kb. Including promoters the combined length of these genes is about 2.9 (XD) plus 2.4 (XK), or about 5.3 kb (*see also* App. Br. 10-11).

19. The smallest plasmid used by Lopes to integrate exogenous DNA into the yeast chromosomal rDNA locus is pMIRY1, which is 6.1 kb (Lopes 469).

20. Thus, not including the xylose reductase gene, combining the XD and XK genes with Lopes' smallest plasmid results in a plasmid ranging in size from 10.1 kb (promoterless XD plus XK plus pMIRY1) to 11.4 kb (promoter-containing XD plus XK plus pMIRY1).

ANALYSIS -- CLAIM 29

We agree with Appellants that the Examiner has not established a prima facie case that claim 29 would have been obvious to a person of ordinary skill in the art in view of Ho and Lopes. As discussed above, Lopes discloses that stable maintenance of its plasmid "is only observed when the complete plasmid has a size no larger than that of the rDNA unit (9.1 kb)" (Lopes 467 (abstract) (FF 13)), and that "[e]ven lengthening pMIRY2 by 0.4 kb . . . results in reduction of stability (Lopes 437 (FF 14)).

In contrast, even assuming the absence of promoters, and the absence of the xylose reductase gene required by claim 29, the combination of Lopes' smallest plasmid with two of the three genes recited in claim 29 is 10.1 kb (FF 20), which is larger than the plasmid size stated by Lopes as being stable (*see also* App. Br. 11). We therefore agree with Appellants that one of ordinary skill in the art would not have been prompted to include all three of Ho's xylose-metabolizing genes in a single plasmid when using Lopes' rDNA integrating sequences to deliver the genes to yeast.

We therefore also agree with Appellants that the Examiner has failed to establish a prima facie case of obviousness for claim 29. Claim 17 also recites that "the exogenous DNA includes genes encoding xylose reductase,

xylitol dehydrogenase, and xylulokinase." Therefore, for the same reasons applied regarding claim 29, we conclude that he Examiner has not established a prima case of obviousness with respect to claim 17.

The Examiner argues:

> It is noted that claim 17 recites genes encoding XR, XD and XK, however, note that the first selection marker could be all three or any one of the three based on the present claim language. Note also that Ho et al. disclose recombinant yeasts containing genes encoding XR, XD and XK, thus the appellant's statement that the combination of the references might result in reduced mitot[ic] stability is not persuasive.

(Ans. 7.)

We are not persuaded by this argument. As discussed above, Lopes discloses that even small increases in size significantly affect the stability of Lopes' plasmid. Thus, we do not agree with the Examiner that one of ordinary skill in the art would have been prompted to include all three of Ho's xylose-metabolizing genes in a single plasmid when using Lopes' rDNA integrating sequences to deliver the genes to yeast.

We therefore reverse the Examiner's obviousness rejection of claims 17 and 29.

OBVIOUSNESS -- HO AND HALLBORN

ISSUE

Claims 28, 29, and 34 stand rejected under 35 U.S.C. § 103(a) as obvious over Ho and Hallborn (Ans. 7-13). Relying on Ho for the teachings discussed above, the Examiner states:

> In-so-far-as Ho et al. does not explicitly teach the integration at multiple sites, Hallborn et al. teach recombinant yeasts that ferments xylose to ethanol, having genes integrated (multiple copies) into the yeast chromosome. The genes taught by

> Hallborn et al. encode xylose reductase and xylitol dehydrogenase. Hallborn et al. also teach yeast from Saccharomyces and a method of transforming cells with integrative plasmids. Additionally, the vector taught by Hallborn et al. autonomously replicates multiple copy plasmids (see pages 1-9).

(Ans. 8.)

Based on the references' teachings, the Examiner concludes that one of ordinary skill in the art would have considered it obvious "to arrive at the claimed invention as a whole because Ho et al. and Hallborn et al. teach the fermentation of sugars to ethanol (i.e. xylose and glucose) using the same yeast strain" (*id.* at 8-9). The Examiner finds that a person of ordinary skill would have been "motivated to combine the teachings of the references because Ho et al. disclose that ethanol is an ideal liquid fuel for automobiles and Hallborn et al. disclose a method to perform stable transformations over time. Therefore, the claimed invention was *prima facie* obvious" (*id.* at 9).

Appellants contend that the Examiner did not establish a prima facie case of obviousness because the combined disclosures of the two references do not teach or suggest all of the limitations in the claims (App. Br. 12-15), and because the references failed to provide motivation and a reasonable expectation of success (*id.* at 15-17).

The issue with respect to this rejection, then, is whether the Examiner has established a prima facie case that one of ordinary skill in the art would have considered claims 28, 29, and 34 obvious in view of Ho and Hallborn.

FINDINGS OF FACT

21. Each of claims 28, 29, and 34 recites a plasmid vector. Each of those claims requires the plasmid to have "a functional yeast autonomous replicating sequence" and exogenous DNA.

22. Claims 28 and 34 require the exogenous DNA to be "flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of a target yeast cell." Claim 29 requires the exogenous DNA to be "flanked on each end by a DNA flanking sequence which is homologous to a reiterated DNA sequence of a target yeast cell."

23. As discussed above, Ho discloses a plasmid having a functional yeast autonomous replicating sequence and exogenous DNA (FF 5-7).

24. As also discussed above, Ho does not disclose that its plasmids contain exogenous DNA flanked by sequences homologous to the ribosomal DNA of a target yeast cell.

25. Hallborn discloses "recombinant yeast strains transformed with xylose reductase and/or xylitol dehydrogenase enzyme genes" (Hallborn 1). Hallborn states that "[a] yeast strain transformed with the xylose reductase gene is capable of reducing xylose to xylitol and consequently of producing xylitol *in vivo*. If both of these genes are transformed into a yeast strain, the resultant strain is capable of producing ethanol on xylose containing medium during fermentation" (*id.*).

26. Hallborn discloses that the gene encoding xylose reductase (XR) can be introduced into yeast using a plasmid "capable of replicating autonomously when transformed into the recipient yeast strain" (Hallborn 7).

27. Hallborn discloses:

> Alternatively, the gene coding for XR can also be integrated into the yeast chromosome, into the ribosomal RNA locus, for instance. For this purpose the ribosomal sequences of a suitable plasmid, eg. plasmid pIRL9 are released, and cloned appropriately to BS+ vector. The gene coding for XR, coupled in between suitable yeast promoter and terminator regions, is

> released from the hybrid vector comprising the gene and cloned into the plasmid obtained at the previous stage. From this resulting plasmid the expression cassette, flanked by ribosomal sequences can be released. This fragment is cotransformed into a yeast with an autonomously replicating plasmid carrying a suitable marker for transformation. The plasmid can be later on removed from the cells containing the xrd gene integrated in the chromosome by cultivating the cells in non-selective conditions. This way, recombinant strains can be obtained which carry no extra foreign DNA such as bacterial vector sequences. If a polyploid yeast strain, such as VTT-A-63015, is used the gene can be integrated also to an essential locus such as the PGKI or the ADH1 locus.

(Hallborn 7.)

ANALYSIS

We agree with Appellants that the Examiner has not made a prima facie case of obviousness for claims 28, 29, and 34. Each of claims 28, 29, and 34 recites a plasmid having both a yeast autonomous replicating sequence and exogenous DNA flanked by DNA sequences homologous to reiterated sequences in a target yeast cell (FF 21, 22).

While Hallborn discloses a DNA vector having the XR gene "flanked by ribosomal sequences" (Hallborn 7 (FF 27)), that construct is not circularized or placed into a plasmid, but is instead is used as a "fragment . . . cotransformed into a yeast with an autonomously replicating plasmid carrying a suitable marker for transformation" (*id.*). Thus, rather than suggesting the desirability of placing the rDNA sequences within an autonomously replicating plasmid, as required by claims 28, 29, and 34, Hallborn teaches that the reiterated sequences homologous to the yeast host should be kept separate from the autonomously replicating plasmid used to cotransform the yeast.

Because Hallborn teaches that its integrating sequences should be kept separate from the autonomously replicating plasmid, we do not agree with the Examiner that one of ordinary skill would have been prompted to add those sequences to the plasmids of Ho. We therefore agree with Appellants that the Examiner has not made a prima facie case that the plasmids recited in claims 28, 29, and 34 would have been obvious to one of ordinary skill in the art in view of Ho and Hallborn.

We therefore reverse the Examiner's obviousness rejection of claims 28, 29, and 34 as obvious over Ho and Halborn.

SUMMARY

We affirm the Examiner's rejection of claims 14-16, 18, 28, 30, and 32-34 as obvious over Ho and Lopes.

We reverse the Examiner's rejection of claims 17 and 29 as obvious over Ho and Hopes.

We reverse the Examiner's rejection of claims 28, 29, and 34 as obvious over Ho and Hallborn.

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a)(1)(iv).

AFFIRMED-IN-PART

dm

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